

IN THE CLAIMS:

1. (Currently amended) A composition comprising a membrane having bound carboxy-blocked phosphoprotein, wherein the phosphoprotein is coordinated to a phosphoprotein detection reagent (PPDR) comprising:
 - (i) a polydentate chelator coordinated to a metal ion selected from the group consisting of Fe^{3+} , Al^{3+} , Yb^{3+} , and Ga^{3+} ; and
 - (ii) a detectable moiety conjugated to the polydentate chelator at a site other than a potential metal ion coordination site;wherein the chelated metal ion selectively binds to a phosphorylated amino acid residue in the phosphoprotein to create a chelator-metal ion-phosphoprotein (CMPP) complex; and the detectable moiety allows the CMPP complex to be detected.
2. (Previously presented) The composition of claim 1, wherein the PPDR is soluble in an aqueous medium.
3. (Previously presented) The composition of claim 1, wherein the chelator is a tetradentate nitriloacetic acid.
4. (Previously presented) The composition of claim 1, wherein the chelator is a tridentate iminodiacetic acid.
5. (Canceled)
6. (Previously presented) The composition of claim 1, wherein the metal ion is Ga^{3+} .
7. (Previously presented) The composition of claim 1, wherein the metal ion is Fe^{3+} .
8. (Previously presented) The composition of claim 1, wherein the detectable moiety is biotin.

9. (Previously presented) The composition of claim 1, further comprising a spacer between the chelator-metal ion moiety and the detectable moiety.

10. (Currently amended) A method for preparing a composition comprising a membrane having bound carboxy-blocked phosphoprotein, wherein the phosphoprotein is coordinated to a phosphoprotein detection reagent (PPDR), the method comprising:

- (a) reacting a polydentate chelator donor molecule with a detectable moiety donor under conditions wherein a detectable moiety is transferred to a polydentate chelator at a site other than a coordination site to form a chelator-detectable moiety complex;
- (b) chelating a metal ion selected from the group consisting of Fe^{3+} , Al^{3+} , Yb^{3+} , and Ga^{3+} to the polydentate chelator to form a PPDR, wherein the PPDR is soluble in aqueous medium; and
- (c) contacting a membrane having bound carboxy-blocked phosphoprotein with the PPDR.

11. (Original) The method of claim 10, wherein the chelator donor molecule is selected from the group consisting of 2-(aminooxyethyl)iminodiacetic acid (AIDA), aminobutyl-nitriloacetic acid (AB-NTA), and iminodiacetic acid (IDA).

12. (Original) The method of claim 10, wherein the detectable moiety donor is selected from the group consisting of sulfo-N-hydroxysuccinimidyl-biotin (sulfo-NHS-biotin), sulfosuccinimidyl-6-(biotinamido) hexanoate (sulfo-NHS-LC-biotin), sulfosuccinimidyl-6-(biotinamido)-6-hexanimido hexanoate (sulfo-NHS-LC-LC-biotin), and penta-fluorophenyl-biotin.

13. (Original) The method of claim 10, wherein the detectable moiety donor is present in the reacting step in a molar excess over the polydentate chelator donor molecule.

14. (Previously presented) The method of claim 10, wherein the chelator-detectable moiety complex and a metal ion-containing solution are present in equimolar concentrations in the chelating step.

15-35. (Canceled)

36. (Currently amended) A kit comprising:

(a) a phosphoprotein detection reagent (PPDR) comprising:

- (i) a polydentate chelator coordinated to a metal ion selected from the group consisting of Fe^{3+} , Al^{3+} , Yb^{3+} , and Ga^{3+} ; and
- (ii) a detectable moiety conjugated to the polydentate chelator at a site other than a potential metal ion coordination site,

wherein the chelated metal ion selectively binds to a phosphorylated amino acid residue in a phosphoprotein if present to create a chelator-metal ion-phosphoprotein (CMPP) complex, and the detectable moiety allows the CMPP complex to be detected if present;

(b) a membrane; and

(c) a carboxy-blocking reagent, and

(d) instructions for using the PPDR.

37. (Canceled)

38. (Original) The kit of claim 36, further comprising a secondary reagent for detecting the PPDR.

39. (Previously presented) The kit of claim 36, wherein the phosphoprotein detection reagent (PPDR) is soluble in aqueous medium.

40. (Canceled)

41. (Currently amended) A composition comprising a membrane having bound carboxy-blocked phosphoprotein, wherein the phosphoprotein is coordinated to a phosphoprotein detection reagent (PPDR) comprising a chelator and a detectable moiety conjugated to the chelator in a binding solution with a pH ranging from about 5.0 to about 7.0, wherein:
- (i) the chelator comprises a tetradentate nitriloacetic acid or a tridentate iminodiacetic acid coordinated to a metal ion selected from the group consisting of Fe^{3+} , Al^{3+} , Yb^{3+} , and Ga^{3+} ;
 - (ii) the chelated metal ion selectively binds to a phosphorylated amino acid residue in the phosphoprotein to create a chelator-metal ion-phosphoprotein (CMPP) complex, and the detectable moiety allows the CMPP complex to be detected; and
 - (iii) the PPDR is soluble in aqueous medium.
42. (Previously presented) The composition of claim 41, wherein the metal ion is Ga^{3+} .
43. (Previously presented) The composition of claim 41, wherein the metal ion is Fe^{3+} .
44. (Previously presented) The composition of claim 41, wherein the detectable moiety is biotin.
45. (Previously presented) The composition of claim 41, further comprising a spacer between the chelator and the detectable moiety.
46. (Currently amended) A composition comprising:
- (a) a membrane;
 - (b) a carboxy-blocked phosphoprotein bound to the membrane;
 - (c) a metal ion selected from the group consisting of Fe^{3+} , Al^{3+} , Yb^{3+} , and Ga^{3+} ;

- (d) a phosphoprotein detection reagent (PPDR) comprising a chelator and a detectable moiety, wherein:
 - (i) the detectable moiety is conjugated to the chelator at a site other than a potential metal ion coordination site;
 - (ii) the chelator comprises a polydentate chelator coordinated to the metal ion to form a chelator-metal ion moiety;
 - (iii) the chelator-metal ion moiety selectively binds to a phosphorylated amino acid residue in the phosphoprotein to create a chelator-metal ion-phosphoprotein (CMPP) complex; and
 - (iv) the detectable moiety allows the CMPP complex to be detected #; and
- (c) a binding solution having a pH ranging from about 5.0 to about 7.0, wherein the chelated metal ion selectively binds to the phosphorylated amino acid residue in the phosphoprotein in the binding solution.

47. (Previously presented) The kit of claim 36, wherein the kit further comprises a binding solution having a pH ranging from about 5.0 to about 7.0.

48. (Previously presented) The composition of claim 1, comprising a binding solution with a pH ranging from about 5.0 to about 7.0

49. (New) The method of claim 10, wherein the membrane having bound carboxy-blocked phosphoprotein is prepared by contacting a membrane having bound phosphoprotein with one of the groups consisting of methanolic HCl, a carbodiimide, and Woodward's Reagent "K."